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THE DIFFERENTIATION OF THE PARATYPHOID-ENTERITIDIS GROUP, III

THE UNCOMMONNESS OF B. SUIPESTIFER IN THE INTESTINES OF NORMAL SWINE

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Observations on the occurrence of bacilli of the paratyphoid-enteritidis-suipestifer group in the intestines of normal animals have been made by several European investigators, but the results and conclusions are widely at variance. The facts regarding the distribution and normal habitat of the suipestifer organisms are practically important in view of their bearing on a variety of human and animal diseases, and especially is this true if the conclusions of certain German writers in regard to the regional distribution of the group be accepted. Since the relations of these organisms to hog cholera are still obscure, the relative frequency of occurrence in normal swine offers another point of interest.

Dorset and his co-workers[1] found in one case in the intestines of normal hogs "an organism which possessed the cultural characters of B. cholerae suis," but which lacked pathogenic power for guinea-pigs. Morgan[2] also reported finding organisms having the cultural and agglutinative reactions of B. paratyphosus A and B. enteritidis in pigs' feces and in the scrapings from the mucous surface of the large and small intestines of these animals. A most comprehensive investigation of the subject was made by Uhlenhuth, Xylander, Hübener and Bohtz,[3] who examined the intestinal contents of 600 presumably healthy swine at the central abattoir in Berlin. These investigators found in 51 of these animals (8.4%) bacilli that resembled in cultural, agglutinative and pathogenic properties the bacilli that they had isolated from animals suffering from hog cholera. These observations have been extensively quoted and have been regarded as explaining the frequent occurrence of these bacilli in hog cholera as a secondary invasion of the

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[1] Dorset, Bolton and McBryde, "Etiology of Hog Cholera," Bull. 72, Bur. of Animal Indus., 1905.

[2] Brit. Med. Jour., 1905, 1, p. 1257.

[3] Arb. a. d. k. Gsndhtsamte, 1907-1908, 27, p. 425.

tissues in the wake of the filterable virus. Observations by other workers have seemed to confirm this view. Grabert[4] reported finding bacilli resembling B. suipestifer in the intestines of 7 out of 23 swine without characteristic intestinal lesions, but the identification tests that he employed do not seem decisive.

Eckert[5] also isolated organisms that he considered to belong to this group from 4 out of 10 normal animals. Seiffert[6] examined the intestinal contents of 60 normal swine (Frankfurt) and found 2 cultures which "fulfilled all conditions for identification with paratyphoid-B bacilli." Schmidt[7] found bacilli "culturally similar to paratyphoid-B bacilli" in about 4% of normal swine feces (700 animals). Only one strain, however, agglutinated up to the titer limit (4,000) of the test serum ("hog cholera serum"). Other strains showed only a slight degree of agglutinability (1:200-1:800) after a number of hours in the incubator, sometimes not before 24 hours. The majority of these feebly agglutinable strains produced indol. Full details of the cultural tests relied on for differentiation are not given in this paper. Velzen[8] and Gardenghi[9] are also said to have found B. suipestifer in normal swine, but I have not been able to see their original papers for details.

Opposed to these findings is the work of Savage[10] who in a careful examination of the intestinal contents of 6 pigs failed to find organisms that could be certainly identified with true paratyphoid-enteritidis bacilli. Aumann[11] likewise obtained negative results from an examination of the intestinal contents of 101 hogs (Hamburg). Aumann states: "Upon serological examination I have found no strain which has shown even the slightest reaction toward our sera." Sobernheim[12] examined 102 samples from the intestines of 51 swine (Berlin) without a positive finding, although in 3 instances organisms which he identified as paratyphoid bacilli were isolated from the liver or spleen of the animals.

My own observations have extended over several years. The swine from which the samples were obtained were all healthy, freshly slaughtered animals in the Union Stock Yards, Chicago. The hogs are shipped in to Chicago from many points throughout the middle west and northwest states (about 50% from Iowa, 25% from Illinois and the remainder chiefly from Minnesota, Wisconsin, South Dakota and Indiana; some come also from Missouri and Michigan and a few from more distant points).

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[4] Ztschr. f. Infektionskr. d. Haustiere, 1907-1908, 3, p. 218.

[5] Inaug. Diss., Giessen, 1909, cited by Hübener, Fleischvergiftungen u. Paratyphusinfektionen, Jena, 1910, p. 67.

[6] Ztschr. f. Hyg., 1909, 63, p. 273.

[7] München. med. Wchnschr., 1911, 58, p. 563.

[8] Diss. Berne, abstr. in Centralbl. f. Bakteriol., I, Ref., 1907-1908, 40, p. 802.

[9] Lo Sperimentale, 1906, abstr. in Centralbl. f. Bakteriol., I, Ref. 1907-1908, 40, p. 807.

[10] Rept. of Med. Off. to Loc. Gov't. Board, 1906-1907, p. 253.

[11] Centralbl. f. Bakteriol., I, O., 1910-1911, 57, p. 310.

[12] Centralbl. f. Bakteriol., I., Ref., Supplement, 1910, 47, p. 170.

The samples were obtained from the lower intestine by slitting the intestinal wall with a sharp sterile knife in the manner described by Uhlenhuth.[13] The usual precautions against contamination were taken.

In the first series, obtained between Oct. 28, 1913, and April 7, 1914, the intestinal contents of 58 hogs were examined. Each sample was examined (a) by direct plating on Endo medium and (b) by preliminary incubation in brilliant green medium. The brilliant green broth was made up according to Torrey's method.[14] About 1 gm. of the intestinal contents from each hog was placed in a tube (10 c.c.) of the medium, shaken thoroughly and allowed to stand for a few minutes. From this 5 other tubes with vials to show gas production were inoculated respectively with 1 c.c. (1), 0.5 c.c. (2), 2-3 drops (2), and incubated for 24-48 hours. Those showing a positive reaction (gas production and darkening of medium) were at once plated out on Endo medium. A control tube of the medium was always carried with the others. Colonies from the Endo plates, direct (a) and after brilliant green broth incubation (b), were transferred to Russell's medium and those strains giving a paratyphoid-like reaction subjected to further study.

In all 725 colonies were transferred to Russell's medium, 343 from Endo medium direct (a) and 382 after brilliant green broth incubation (b). Of these 215 [98 (a) and 117 (b)] were inoculated into dextrose and lactose broths and gelatin. The majority of these proved of the B. coli type [151; 81 method (a), 70 method (b)], but the brilliant green incubation let through also a number of bacilli apparently of the B. proteus group [41, method (b)]. Thirteen strains were of uncertain affinities, while 10 from 6 different animals [9 isolated by method (a), 1 by method (b)] gave reactions in these media corresponding with those of the B. suipestifer type.* These 10 strains were then tested further. Only 4 of these gave milk reactions exactly corresponding to those of the paratyphoid group. None of them fermented saccharose, in this respect also resembling B. suipestifer.

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[13] Arb. a. d. k. Gsndhtsamte, 1907-1908, 27, p. 425.

[14] Jour. Infect. Dis., 1913, 13, p. 263.

* Dextrose — ; lactose — ; gelatin, not liquefied.

Dulcit was not fermented by any of the strains, but this is not a diagnostic character since, as I have previously shown,[15] the majority of typical suipestifer strains attack this carbohydrate tardily or not at all. On the other hand, only two of these fermented rhamnose and only two sorbit, one fermented salicin, and all but three produced indol. Not one of the 10 strains corresponded in cultural and biochemical characters with the suipestifer or paratyphosus type as described in a previous paper.[15] Agglutination tests on all these organisms were made with B. paratyphosus B serums (3 strains), B. enteritidis (1 strain), and B. suipestifer (2 strains). In no case has more than a trace of agglutination at 1:100 dilution been shown, although serums with high titers (5000-10,000, one B. suipestifer serum of over 20,000) have been employed. Not a single culture in the series of 725 colonies from 58 different swine can be regarded as belonging to the suipestifer or paratyphoid type.

In the second series, March 7-May 2, 1917, specimens from 100 swine were examined. The samples of intestinal contents were obtained in the same way and from the same source (freshly slaughtered hogs in the Union Stock Yards, Chicago) as before. Endo medium alone was used in this series. Three plates of three different dilutions were made from each specimen. Paratyphoid-like colonies were transferred to agar tubes and thence inoculated into dextrose and lactose broths, litmus milk and gelatin as before. Two hundred and fifty-three colonies were examined in this way. Twelve from eleven different animals gave reactions in dextrose and lactose broths, and in gelatin corresponding with those of the B. paratyphosus B type. Eight of these, however, gave reactions in litmus milk (30 days) that were unmistakably not of the Para B or Para A type. All but one produced indol; several fermented salicin, and others failed to ferment either rhamnose or sorbit or both.

One strain resembled the suipestifer type in nearly all of its cultural characters, but failed to ferment sorbit. All the authentic members of the suipestifer group that I have tested give a positive result with this carbohydrate.[15] This strain did not agglutinate in 1:100 dilutions with potent serums of the paratyphosus A (4), paratyphosus B (12), suipestifer (63), or enteritidis (52) types.

The third series, June 14-Sept. 22, 1917, included examinations of the intestinal contents from 133 swine from the same source as before.

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[15] Jordan: Jour. Infect. Dis., 1917, 20, p. 457.

The methods used were those employed in the second series. Four hundred and forty-one colonies were picked and tested. Eighteen of these from 13 different animals were dextrose +, lactose —, and failed to liquefy gelatin. None of these, however, corresponded in all their cultural characters with the suipestifer type. The lack of correlation of cultural characters in these strains is illustrated in the accompanying table.

TABLE 1

ILLUSTRATING THE CULTURAL DEVIATIONS OF STRAINS TESTED WITH 5 DIFFERENT AGENTS. THE SPECIMENS WERE TAKEN FROM THE THIRD SERIES OF ANIMALS

Number	Typical Reaction Milk	Indol	Salicin	Sorbit	Rhamnose
Suipestifer type * (63)	+	—	—	+	+
P 109 D	—	+	—	+	+
P 111 A	—	—	—	+	—
P 111 D	—	—	—	+	—
P 111 E	—	—	—	+	—
P 119 B	—	+	—	+	+
P 124 D	—	+	—	—	—
P 129 D	—	+	—	—	—
P 141 A	—	—	+	+	+
P 160 A	+	+	+	+	+
P 160 D	+	+	+	+	+
P 187 E	—	—	+	+	+
P 192 A	—	—	+	—	+
P 198 C	—	+	—	—	—
P 216 B	—	—	—	—	—
P 219 A	—	+	—	+	+
P 219 B	—	+	—	+	+
P 223 A	+	—	+	—	—
P 223 B	+	—	+	—	—

From the results recorded in the table it would seem not unlikely that all the mathematically possible combinations would occur in a sufficiently large series. Although only salicin, sorbit and rhamnose reactions are shown in Table 1, I have evidence that the use of other carbohydrates would increase the extent of deviation from the suipestifer type of fermentation. Some of these strains are maltose negative and behave in other ways differently from the true paratyphosus-suipestifer types.

SUMMARY AND CONCLUSIONS

In all, 1419 strains from the lower intestine of 291 normal swine have been tested. Only 40 of these have proved to be dextrose +, lactose —, and unable to liquefy gelatin.

Using only the 5 tests indicated in the accompanying table, 7 out of 40 tested gave the suipestifer reaction in milk,† 14 out of 40 did not produce indol, 22 out of 32 did not ferment salicin, 14 out of 32 fermented sorbit, and 11 out of 25 fermented rhamnose. In no instance were more than three of these five characters of the suipestifer type united in one strain. Of the 26 strains closest to the true type, 9 differed in two of the three differential tests, 7 in three and 9 in four. These strains have also been tested with paratyphoid B, suipestifer and enteritidis serums sufficiently potent to agglutinate the homologous organisms in 5,000-10,000 dilution, but not one has ever shown more than a trace of agglutination in 1: 100 dilution.

It must be concluded, therefore, that the occurrence of true suipestifer strains in any abundance in the intestines of normal swine in this country is a rarity. The alleged frequency of occurrence of these bacilli reported from some places in Europe may perhaps be due to the inclusion of a high proportion of hogs that had become permanent or temporary carriers through their association with infected animals. Perhaps in some instances also, identification has been made on the basis of too few biologic and cultural characters. Some writers describe indol-producing strains as if they were to be differentiated hardly or not at all from typical members of the group. In the course of my examination in the past 4 years of some hundreds of typical and atypical suipestifer and paratyphoid bacilli, I have never yet found an indol-producing bacillus that did not differ in some other cultural respect or that agglutinated to any degree showing a biologic relationship with the type organisms. The observations recorded in this paper suggest that the true B. suipestifer is not a common inhabitant of the intestine of normal swine, and that its occurrence in these animals is to be looked upon as an expression of the "carrier" condition.

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† Including slow production of alkalinity as in the paratyphosus-A type.